Journal of Surgical Oncology 1999;70:230–234

Prognostic Impact of Cyclin-Dependent Kinase Inhibitor $p27^{kip1}$ in Node-Positive Breast Cancer

ATSUO TSUCHIYA, MD,* GUO JUN ZHANG, MD, AND MASAHIKO KANNO, MD
Department of Surgery II, Fukushima Medical University School of Medicine, Fukushima, Japan

Background and Objectives: $p27^{kip1}$ (p27) plays an important role as a negative regulator of cell cycle-dependent kinase activity during progression of the cell cycle. The most important prognosticator of breast cancer is nodal status, and the aim of this study was to determine the prognostic implication of p27 in breast cancer patients with lymph node metastases.
Methods: Immunohistochemical staining for p27 was performed on tissues from 102 patients with node-positive breast cancer.
Results: A nuclear staining over 50% was defined as high expression. High expression of p27 was shown in 59 patients (57.8%). A significant correlation was found between high p27 and positive estrogen receptor status, but there was no correlation between p27 staining and age, menopausal status, nodal status, or tumor size. Low expression of p27 was significantly associated with shorter survival. A multivariate analysis also showed that the only independent variable was p27.
Conclusions: The results indicated that low expression of p27 was an independent factor associated with poor prognosis. Therefore, p27 can be an important tool in making therapeutic decisions.
J. Surg. Oncol. 1999;70:230–234.

KEY WORDS: breast cancer; p27; survival; prognostic factor

INTRODUCTION

Cell cycle progression is regulated by the combined action of cyclins, cyclin-dependent kinases (cdk), and cdk inhibitors. The activity of cdk is regulated by the phosphorylation by cdk-activation kinase and by the action of certain inhibitory proteins called cdk inhibitors. A cyclin-dependent kinase inhibitor, $p27^{kip1}$ (p27), regulates progression from G1 into S phase by binding to and inhibiting the cyclin E/cdk2 complex. p27 protein is present in quiescent cells, and levels decrease when cells are stimulated by growth factors [1].

The most important prognosticator of breast cancer is nodal status [2]. The proliferative activity of breast cancer cells is well correlated to their biological behavior, and kinetic information can provide useful prognostic information in the treatment of cancer. In addition to hormone receptors, cell proliferation and other biomarkers are now receiving considerable attention, and preliminary results on their roles as predictors of therapeutic response are encouraging [3]. Therefore, these indices may be useful in determining which patients would benefit from more aggressive therapy.

Recent reports claimed that p27 staining by immunohistochemistry can be performed on routine formalin-fixed, paraffin-embedded specimens of various neoplasms, and the preliminary results, which indicated that reduced p27 expression may be a significant predictor of poor survival in various tumors, seem to be promising [4–8]. The aim of this study was to investigate a possible correlation between p27 expression and the clinicopathologic features of patients with lymph node-positive primary breast cancer and to evaluate the prognostic impact of p27.

*Correspondence to: Atsuo Tsuchiya, MD, Department of Surgery II, Fukushima Medical University School of Medicine, 1, Hikarigaoka, Fukushima 960-1295, Japan. Fax No.: (81) 24-548-3249. E-mail: hazuki@cc.fmu.ac.jp
Accepted 2 January 1999

PATIENTS AND METHODS

Patients

A total of 102 female breast cancer patients with lymph node metastases who were operated on in Fukushima Medical University Hospital between 1986 and 1991 were eligible. Eligibility criteria were histologic diagnosis of invasive breast cancer, axillary lymph node metastases, and no distant metastasis at the first diagnosis. The mean age of the patients was 52.4 years, ranging from 26 to 83 years. These patients were followed up clinically and histologic breast cancer tissue was investigated immunohistochemically. All of the patients postoperatively received endocrine therapy and/or chemotherapy. Postoperative follow-up data were obtained by periodic examinations at the outpatients' clinic or from mailed questionnaires.

Immunohistochemistry

Tissue samples fixed in 10% formalin and embedded in paraffin were cut into 4 μm sections. p27 was detected with the monoclonal antibody $p27^{kip1}$ (Transduction Lab., Lexington, KY). Tissue localization of p27 was determined immunohistochemically by the avidin-biotin-peroxidase complex (ABC) method. Sections were treated with 0.1 mol/liter citrate buffer, pH 6.0, in a 650 Watt microwave oven for 10 min for antigen retrieval before immunostaining. The primary antibody was diluted at 1:500 in 0.05 mol/l phosphate-buffered saline overnight at 4°C. The sections were reacted with biotin-labeled goat antibody IgG and incubated with ABC (Vector Lab., Burlingame, CA). Diaminobenzidine substrate was then added and sections were counterstained with hematoxylin, dehydrated, and mounted. At least 10 high-power fields were chosen and scored for the percentage of cells showing nuclear p27 staining. A nuclear staining over 50% was defined as high and below 49% as low.

Clinicopathologic Variables

The following clinicopathologic variables were studied: menopausal status (pre-, post-), number of positive lymph nodes (1–3, ≥4), tumor size (≤2 cm, ≥2.1 cm), and estrogen receptor (ER) status. ER was measured according to the dextran-coated charcoal method [9]. All results were expressed in femtomoles per milligram (fmol/mg) of cytosol protein, with 5 fmol/mg protein being the cutoff level.

Statistical Methods

Kaplan-Meier survival curves of time to recurrence and death [disease-free survival (DFS), overall survival (OS)] were analyzed by the generalized Wilcoxon test and log-rank test. The association between the various clinicopathologic factors and p27 expression was analyzed using the chi-square test. Cox regression analysis was used to estimate the independent predictive value of the variables. *P* values were based on two-tailed tests, and $P < 0.05$ was considered statistically significant.

TABLE I. Relationship Between p27 Expression and Clinicopathologic Variables in Node-Positive Breast Cancer

Variables	p27—high (n = 59)	p27—low (n = 43)	*P*
Age (years) (mean ± SD)	53.4 ± 12.9	51.0 ± 12.0	0.3334 (NS)[a]
Menopausal status			
Pre-	27	20	0.8744 (NS)
Post-	32	23	
Nodal status			
1–3	38	26	0.6846 (NS)
≥4	21	17	
Tumor size (cm)			
≤2	20	9	0.1215 (NS)
≥2.1	39	34	
ER status[b]			
+	39	17	0.0055
−	16	23	

[a]NS, not significant.
[b]Data on ER status in 7 cases were deficient.

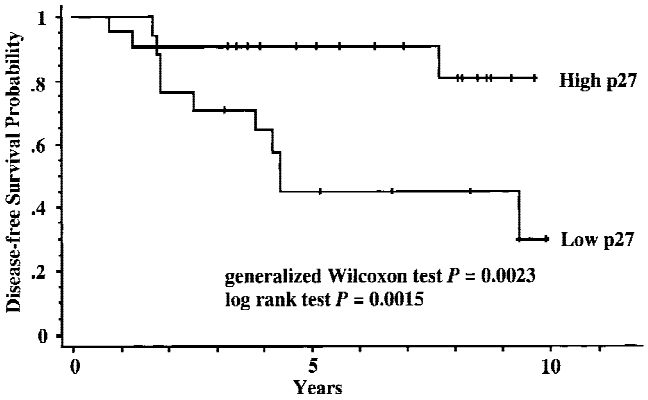

Fig. 1. DFS according to p27 expression.

RESULTS

Immunohistochemistry

High reactivity of p27 was seen in 59 cases (57.8%) and low staining was shown in 43 cases. The correlation between the degree of p27 staining and age, menopausal status, tumor size, and ER status is shown in Table I. A significant correlation was found between high p27 and positive ER status, but there was no correlation between p27 and other variables.

Clinical Outcome of the Patients

Disease relapses were seen in 32 patients. Twenty-six patients died of the disease. Five-year DFS and OS were 74.7% and 69.6%, respectively.

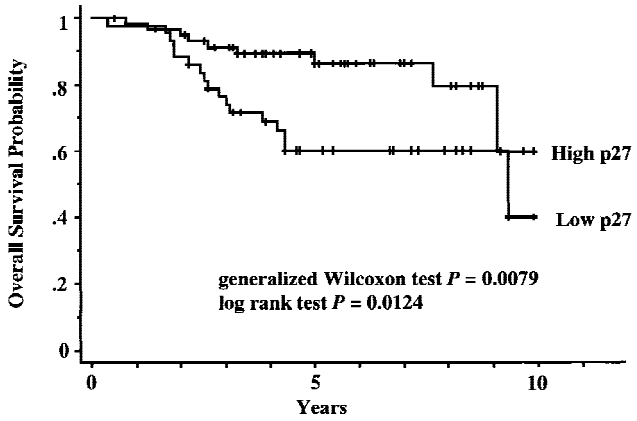

Fig. 2. OS according to p27 expression.

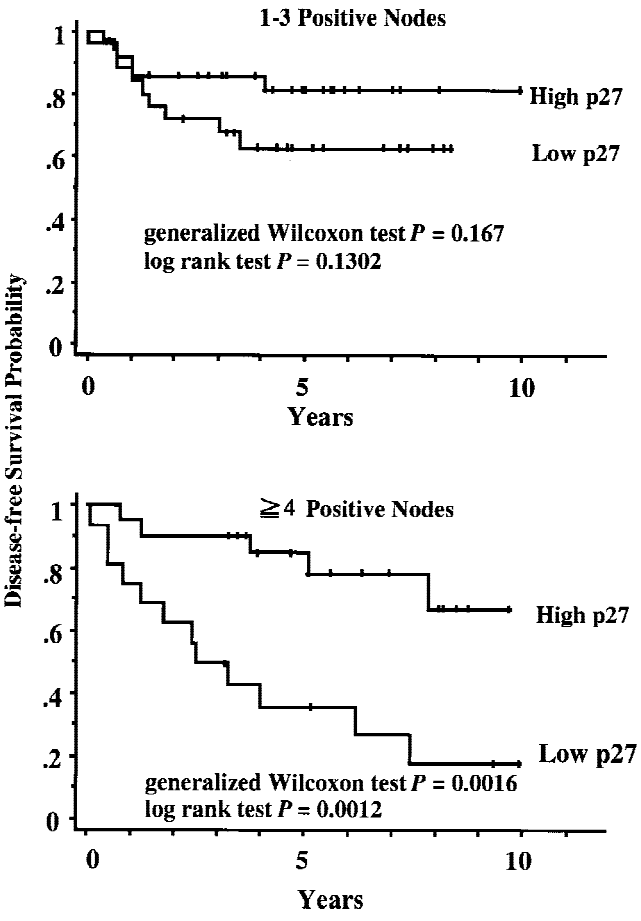

Fig. 3. DFS according to p27 expression. Analysis of the patients with 1–3 **(top)** and 4 or more **(bottom)** involved lymph nodes.

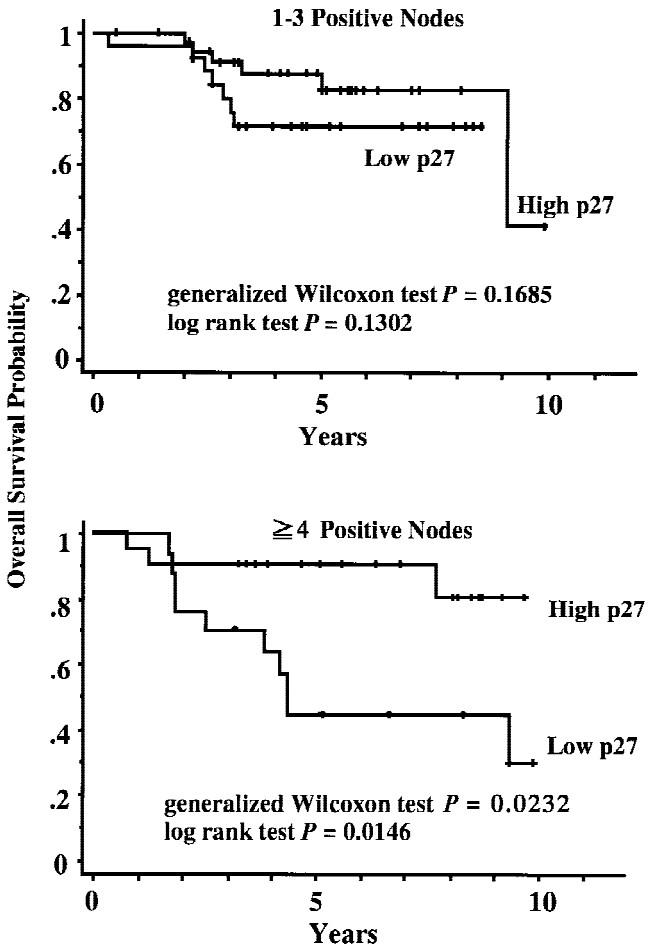

Fig. 4. OS according to p27 expression. Analysis of the patients with 1–3 **(top)** and 4 or more **(bottom)** involved lymph nodes.

Survival Analysis

In the whole series of patients, low expression of p27 was related to shorter DFS and OS (generalized Wilcoxon: $P = 0.0023$, 0.0079; log rank: $P = 0.0015$, 0.0124, respectively) (Figs. 1, 2). The degree of lymph node metastases and tumor size were also related to DFS and OS. Depending upon the number of positive lymph nodes, the patients were divided into two groups: one group with a count of 1–3 and the other group with 4 or more. The patients with low p27 expression had significantly shorter DFS and OS of the group with 4 or more involved lymph nodes (Figs. 3, 4).

Of 102 cases, data on ER status were deficient in 7 cases, and 4 factors (p27, nodal status, tumor size, ER status) of 95 cases were analyzed in a multivariate analysis, which showed that low p27 expression was independently associated with clinical outcome (Table II). The only variable with independent prognostic value for DFS and OS was p27 expression.

DISCUSSION

Members of the cip/kip family of cdk inhibitors bind to cyclin/cdk complexes and block progression through the cell cycle. The cdk inhibitors include p21, p27, p57 in the cip/kip family and p15, p16, p18, and p19 in the ink4

TABLE II. Scoring of Variables and Multivariate Analysis of Prognostic Variables for OS

		Multivariate analysis		
Variables	Scoring	Chi-square	Hazard ratio	*P*
p27	0: high, 1: low	5.26	2.969	0.0218
Nodal status	0: 1–3, 1: ≥4	0.506	1.248	0.256 (NS)[a]
Tumor size	0: ≤2 cm, 1: ≥2.1 cm	0.931	1.805	0.867 (NS)
ER status	0: (+), 1: (−)	0.876	1.482	0.767 (NS)

[a]NS, not significant.

family. Current theories propose p27 to be a central signal that coordinates the varied inputs from the extracellular environment and serves as a threshold for progression to S phase or for exit from the cell cycle. Overexpression of kip protein causes cell cycle arrest. The possible role of p27 as a tumor suppressor gene has been analyzed in a large variety of human tumors and tumor cell lines [10,11]. A p27 mutation found in breast cancer, although rare, may be important for tumorigenesis [11], and loss of p27 expression may be associated with tumor progression [12]. Therefore, it is possible that tumor cells can downregulate the protein expression of p27 to overcome the growth inhibitory activity of p27 during tumor development.

Low p27 expression proved to be a significant predictor of poor survival by multivariate analysis in this study. Porter et al. [5] reported that the combination of p27 and its target protein, cyclin E, had a further prognostic value. A combination of low cyclin E and high p27 expression, indicative of slow progression through G1 of the cell cycle, correlated with about a 70% 10-year OS. Yasui et al. [8] also demonstrated an inverse correlation between the expressions of p27 and cyclin E in gastric carcinoma.

The role of p27 is considered to be the control of cell proliferation through the inhibition of cdk2, but the proliferative index by Ki-67 staining showed no correlation with p27 expression in colorectal carcinoma [7]. Catzavelos et al. [6] also reported the lack of correlation between p27 overexpression and S phase in breast cancer, and speculated that there are mechanisms of action of p27 independent of proliferation which affect tumor progression. High levels of p27 were seen in some highly proliferative breast cancer cells, which is suggestive of the existence of a mechanism by which some growing tumor cells may tolerate this inhibitor of cell cycle progression [13]. On the contrary, Lloyd and associates [14] reported that Ki-67 immunostaining of normal and neoplastic tissue generally showed an inverse pattern of nuclear immunoreactivity compared with p27.

It has been shown that the regulation of the cellular abundance of p27 in the cell cycle occurs at the posttranslational level [15]. These data reflect recent research including findings that posttranslational ubiquitin-mediated proteasomal proteolysis represents the major regulatory influence on p27 protein levels [16]. In addition, p27 has been shown to play a role in adhesion-dependent cell growth [17]. Loss of p27 may confer the ability to grow in a milieu of altered intercellular adhesion and altered extracellular matrix properties, and this could facilitate metastasis.

CONCLUSIONS

The preliminary results of this retrospective investigation show that the low expression of p27 is potentially a useful prognosticator for node-positive breast cancer, and it deserves further prospective investigation.

REFERENCES

1. Kato J, Matsuoka M, Polyak K, et al.: Cyclic AMP-induced G1 phase arrest mediated by an inhibitor($p27^{kip1}$) of cyclin-dependent kinase 4 activation. Cell 1994;79:487–496.
2. Fisher B, Redmond C, Fisher ER, et al.: The contribution of recent NSABP clinical trials of primary breast cancer therapy to an understanding of tumor biology—An overview of findings. Cancer 1980;46:1009–1025.
3. Clark GM: Do we really need prognostic factors for breast cancer? Breast Cancer Res Treat 1996;30:117–126.
4. Tan P, Cady B, Wanner M, et al.: The cell cycle inhibitor p27 is an independent prognostic marker in small ($T_{1a,b}$) invasive breast carcinomas. Cancer Res 1997;57:1259–1263.
5. Porter PL, Malone KE, Heagerty PJ, et al.: Expression of cell-cycle regulators $p27^{kip1}$ and cyclin E, alone and in combination, correlate with survival in young breast cancer patients. Nature Med 1997;3:222–225.
6. Catzavelos C, Bhattacharya N, Ung YC, et al.: Decreased levels of the cell-cycle inhibitor $p27^{kip1}$ protein: Prognostic implications in primary breast cancer. Nature Med 1997;3:227–230.
7. Loda M, Cukor B, Tam SW, et al.: Increased proteasome-dependent degradation of the cyclin-dependent kinase inhibitor p27 in aggressive colorectal carcinomas. Nature Med 1997;3:231–234.
8. Yasui W, Kudo Y, Semba S, et al.: Reduced expression of cyclin-dependent kinase inhibitor $p27^{kip1}$ is associated with advanced stage and invasiveness of gastric carcinoma. Jpn J Cancer Res 1997;88:625–629.
9. McGuire WL, DeLaGarza M: Improved sensitivity in the measurement of estrogen receptor in human breast cancer. J Clin Endocrinol Metab 1973;37:986–989.
10. Kawamata N, Morosetti R, Miller CW, et al.: Molecular analysis of the cyclin-dependent kinase inhibitor gene p27/kip1 in human malignancies. Cancer Res 1995;55:2266–2269.
11. Spirin KS, Simpson JF, Takeuchi S, et al.: p27/kip1 mutation found in breast cancer. Cancer Res 1996;56:2400–2404.
12. Chen J, Willingham T, Shuford M, et al.: Tumor suppression and inhibition of aneuploid cell accumulation in human brain tumor

cells by ectopic overexpression of the cyclin-dependent kinase inhibitor $p27^{kip1}$. J Clin Invest 1996;97:1983–1988.
13. Fredersdorf S, Burns J, Milne AM, et al.: High level expression of $p27^{kip1}$ and cyclin D1 in some human breast cancer cells: Inverse correlation between the expression of $p27^{kip1}$ and degree of malignancy in human breast and colorectal cancers. Proc Natl Acad Sci USA 1997;94:6380–6385.
14. Lloyd RV, Jin L, Qian X, et al.: Aberrant $p27^{kip1}$ expression in endocrine and other tumors. Am J Pathol 1997;150:401–407.
15. Hengst L, Reed SI: Translational control of $p27^{kip1}$ accumulation during the cell cycle. Science 1996;271:1861–1864.
16. Pagano M, Tam SW, Theodoras AM, et al.: Role of the ubiquitin-proteasome pathway in regulation abundance of the cyclin-dependent kinase inhibitor p27. Science 1996;269:682–685.
17. St. Croix B, Flørenes VA, Rak JW, et al.: Impact of the cyclin dependent kinase inhibitor $p27^{kip1}$ on resistance of tumor cells to anticancer agents. Nature Med 1996;2:1204–1210.